

THE EFFECT OF X-RAY ON SOME IMMUNOLOGICAL PARAMETERS AMONG RADIOLOGY TECHNICIANS IN AL-MUTHANNA PROVINCE

A THESIS

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بِسْمِ اللهِ الرَّحْمنِ الرَّحِيمِ

(لَا يُكَلِّفِمُ اللَّهُ نَفْسًا إِلَّا وُسْعَمَا لَمَا مَا كَسَبَتْ وَمَلَيْمَا مَا اكْتَسَبَتْ رَبَّذَا لَا تُوَاخِذْنَا إِنْ نَسِيذَا أَوْ أَخْطَأْذَا رَبَّذَا وَلَا تَحْمِلْ عَلَيْذَا إِحْرًا كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِنْ قَبْلِذَا رَبَّذَا وَلَا تُحَمَّلْنَا مَا لَا طَاقَةَ لَذَا بِهِ وَاعْفِمُ عَذًا وَاعْفِرْ لَذَا وَارْحَمْنَا أَنْتَ مَوْلَاذَا فَانْصُرْنَا عَلَى الْ

حدق الله العلي العظيم

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Declaration

I certify that the thesis entitled (**The Effect of X-ray on Some Immunological Parameters among Radiology Technicians in Al-Muthanna Province**) has been prepared under my supervision in the Department of Biology, college of Science/ AL-Muthanna University as a part of the requirement of the master degree of Sciences in Biology /Microbiology.

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Dedication

I dedicate this humble effort

To who gave me the power to gain this knowledge Allah, Prophet Mohammed and Ahl al-Bayt (peace be upon them).

To the Imam who all people are waiting to appear to make the world as a paradise Imam Al-Mahdi (peace be upon him).

To the people who spend all their life to bring me to this stage of the live, to the lovely couple that I have been never seen in my life at all, to my father and mother.

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And sorry to those I forgot.....

Marwa Hussain Mahal

ABSTRACT

The present study focused on the immune system health for X-ray technicians, since X-ray is a type of ionizing radiation with high energy that produces ionization or electric charges when it passes through matters. Exposure to such type of radiation leads to different cancers and recurrent infections, X-ray technicians are in continuous exposure to this type of IR. Evaluation of their immune system health by estimating some viable parameters that were done for first time at Al-Muthanna governorate in the present study. A total of (89) individuals were involved, (60) cases (X-ray technicians) and (29) healthy people, blood samples were taken during the period extended from December, (2015) to March, (2016) from Al-Muthanna Educational, Gynecology and Pediatric, Al-Rumaytha, Al-Khdir Hospitals and Specialized Dentology Center; concerning the cases that tested. While; controls samples were taken from ordinary healthy peoples in Al-Muthanna governorate community with no history of radiation exposure. The present study investigated parameters of direct link with radiation exposure, included: Total W.B.Cs counts, Differential W.B.Cs counts, Hemoglobin concentrations, NBT Reduction Tests for Neutrophils phagocytic activity detection, and ELISA Tests for IL-2, IL-12 and IL-18 levels estimation. Also; data related to this research investigation were collected using special data form used for recording each case information. The results indicated that X-ray exposure caused suppression in innate immunity and CMI due to disturbance in the parameters related in both types of exposure; acute and chronic; in X-ray technicians. Moreover; recorded data showed poor personal protection equipment PPE outfit for X-ray technicians and weak periodic checkup and monitoring for radiation exposure in Al-Muthanna Governorate.

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List of Abbreviation	
Abbreviation	Terms
3D	3 Dimensional
Ab	Antibody
ADCC	Antibody Dependent Cell-mediated Cytotoxicity
Ags	Antigens
ALARA	As Low As Reasonable Achievable
APC	Antigen Presenting Cell
ATP	Adenosine Tri Phosphate
CD4 ⁺	cluster of differentiation-4
CD8 ⁺	cluster of differentiation-8
СМІ	Cell Mediated Immunity
CNSC	Canadian Nuclear Safety Commission
CT scan	Computed Tomography scan
CTLs	Cytotoxic T-Lymphocytes
D.W	Distilled Water
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked Immune Sorbent Assay
EPA	Environmental Protection Agency
ESR	Erythrocyte Sedimentation Rate
Ev	Electron volt
Gr	Gram
Gy	Gray
Hb	Hemoglobin
HMI	Humoral Mediated Immunity

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ICRP	International Commission on Radiation Protection
IFNs	Interferons
IFN-α	Interferon-alpha
IFN-γ	Interferon-gamma
IL	Interleukin
IR	Ionizing Radiation
kD	kilo Dalton
Kev	Kilo electron volt
М	Mean
MØ	Macrophage
МНС	Major Histocompatibility Complex
Min	Minute
mL	Milliliter
NBT	Nitro Blue Tetrazolium
NCRP	National Concil on Radiation Protection ane measurements
NK cell	Natural Killer
Nm	Nanometer
NRC	Nuclear Regulatory Commission
OD	Optical Density
PBS	Phosphate Buffer Saline
PCV	Packed Cell Volume
Pg	Picogram
Ph	potential Hydrogen
PMNs	Pleomorpho nuclear leukocytes
PPE	Personal Protection Equipment

DDC	
K.B.Cs	Red Blood Cells
r.p.m	Round per minute
Rad	Radiation Absorbed Dose
RNA	Ribonucleic Acid
RT	Room Temperature
SAS	Statistical Analysis System
SE	Standard Error
T _{DTH}	T delayed type hypersensitivity
TCR	T cell Receptor
TGF-β	Transforming Growth Factor- β
Th	T helper
UNSCEAR	United nation scientific committee on the effect of atomic radiation
UV	Ultra Violet
W.B.Cs	White Blood Cells
WHO	World Health Organization
WNO	World Nuclear Organization
μL.	Micro Litter
μsv	Micro Sievert

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CHAPTER ONE INTRODUCTION

1.1 Introduction

Radiation, whether dangerous or helpful has a deep effect on human life. Its contains particles or waves that have enough energy to move through and interact with matter (Bessonov, 2006).

There are two types of radiation, non-ionizing radiation and ionizing radiation. Non-ionizing radiation is considered to be bioactive and harmful. It includes Ultra violet (UV), visible light and electric and magnetic field (CNSC, 2012 and Valko *et al* ., 2004).

In contrast ionizing radiation IR has enough energy to change the chemical structure of atoms and molecules and has the ability to ionize atoms and molecules of matter. This process is known as the ionization, such as X-ray which creates ions by knocking electrons out of their orbits (Brenner &Hall, 2007 and Kitchen, 2001).

Radiation exposure affects organisms in many different ways, X-rays are the form that most people are exposed to outside of solar radiation, the damage of living tissue includes; change in cell structure and DNA damage. The amount of damage depends upon the type of radiation and some cells are more sensitive to radiation. Most of cellular damage can be repaired, but some cells, however may not recover as well as others could become cancerous (EPA, 2012).

1.2 Aims of the study

1. Evaluating the immunological status of X-ray technicians by estimating some parameters related to innate immunity and cell mediated immunity (CMI).

2. Screenning X-ray technicians in Al-Muthanna governorate hospitals to investigate their PPE and periodic check.

CHAPTER TWO LITERATURE REVIEW

2.1The Immune System

The immune system is the defense system that shield our bodies from attacking pathogenic microorganisms and cancers. It can produce an enormous variety of cells and molecules capable of specifically recognizing and eliminating foreign invaders and abnormal cells like mutated cancerous cells. These specific cells and molecules act together in a dynamic system helping each other to achieve their objective (Kuby, 2010).

Functionally, an immune response can be divided into two related activities – recognition and response. The immune system is able to recognize foreign molecules and the body's own cells and protein. This capacity to identify between self and non-self is important to protect the organism from invading pathogens and to eliminate modified or altered cells (e.g. malignant cells), since some pathogens may replicate intracellularly (viruses and some bacteria and parasites) or extracellularly (most bacteria, fungi and parasites) (Abbas *et al.*, 2014).

Once foreign pathogen has been renowned, the immune system uses an appropriate response called an effector response; to clear the organism. The system is able to change the first recognition event into a variety of effector response. After exposure to the similar foreign organism that induced a memory response, an immune response will be generated characterized by rapid and discriminating immune reaction that helps to eliminate the pathogen and prevent disease (Kuby, 2010).

2.1.1 Categories of Immune System

Immunity; the condition of defense from contagious disease, has both a specific and non-specific components. Nonspecific component or innate immunity, supply the first line of defense against foreign microorganisms and prepare before the onset of infection, and their mechanism are not specific to pathogen but include cellular molecular component that recognize classes of molecules which face pathogen (Abbas *et al.*, 2014). Innate immunity is composed of four types of protective barriers including : anatomic, phagocytic, physiologic and inflammatory barriers which provides protection against pathogen. In contrast to the innate immunity, the specific component, adaptive immunity does not come into play until there is an antigenic challenge to the foreign microorganism. Adaptive immunity responds to the challenge with high degree of specificity as well as memory (Kono&Rock, 2008).

There is an adaptive immune response against pathogen within five or six days after first encounter with an antigen. Later exposure to the same antigen results in a memory response, and immune response to the second challenge occur more quickly than the first and more effective in eliminating and clearing the pathogen (Kuby, 2003).

Innate immunity depends mainly on white blood cells or leucocytes (granulocytes and macrophages), while adaptive immune responses depend on lymphocytes, which provide the lifelong immunity that can follow exposure to disease or vaccination. Together, the innate and adaptive immune systems provide an amazing defense system (Bonizzi & Karin, 2004).

2.1.2 Innate Immunity

When microorganisms penetrate the epithelial surfaces; they are usually killed by the innate immune response. Innate immunity recognizes microbes but it does not produce immunological memory (Hoebe *et al.*, 2004).

Macrophages and neutrophils have surface receptors that recognize and bind to many foreign microorganisms. Binding induces engulfment, killing and degradation of pathogen during a process termed phagocytosis. Following phagocytosis, activated macrophages secrete specific molecules or chemical messengers called cytokines, which play major role in communication among immune system cells and participate in inflammation initiation. Inflammation increases blood vessel permeability, rapidly increasing delivery of polymorph nuclear leucocytes (PMNs) cells and inflammatory proteins of the immune system to the affected area (Kuby, 2010).

Activation of plasma proteins called complement system that coat or opsonize microbes increasing the efficiency of phagocytes. Complement also lyses some bacteria, and releases small pro-inflammatory peptides. Macrophage activation results in cytokine release, causing a rise in body temperature and an acute phase response. Acute phase proteins are produced in the liver and contribute to inflammation and host defense (Mosser & Edwards, 2008).

2.1.3 Phagocytosis and Intracellular Killing

Phagocytosis is a very important process during non-specific immune response when specialized cells engulf foreign body like bacteria or molecule like toxin or virus. The phagocytosis process has four steps: Chemotaxis; Endocytosis; Phagolysosome formation and degradation of foreign substances and finally Lysis and excretion. The cells that are able to do phagocytosis are (monocytes, macrophage, PMNs and dendric cells) (Keogan *et al.*, 2006). The results of successful phagocytosis can be either a complete destruction of the foreign body and excretion (concerning phagocytosis done by PMNs), or complete destruction of the foreign body and some parts; (certain polypeptides; of it will be processed and presented on the surface of the phagocytic cells near the MHC molecules(Major Histocompatibility Complex) (concerning phagocytosis done by monocytes, macrophage and dendric cells) then the phagocytic cell will act as antigen presenting cell (APC) (Champion& Mitragotri, 2006).

2.1.4 Innate Immunity Lead to Activation of Adaptive Immune Response

The innate immune response can enhance adaptive immunity according to (Wang *et al.*, 2004) via:

◆ Macrophages enhance the adaptive immune response by acting as antigen presenting cells (APCs).

• Cytokines produced by cells (macrophages and NK cells) of innate immunity enhance responses by the adaptive immune response (e.g. IL-1, IL-6, IL-12 and all IFNs).

• The inflammatory response increases the flow of lymph containing antigen and APCs to the lymphoid tissue.

2.1.5 Adaptive Immune Responses

Adaptive immunity provides protection when innate immunity fails to eliminate an infection. Adaptive immunity develops slowly, has unique specificity for antigen and produces immunological memory. Adaptive immunity results in selection of lymphocyte clones bearing highly antigen-specific receptors (recognition molecules) (Abbas *et al.*, 2014). Each lymphocyte expresses cell-surface receptors of a single specificity, B-cell receptors (immunoglobulins) bind extracellular molecules and pathogens, while T-cell receptors (TCR) bind peptide fragments bound to MHC molecules on cell surfaces (Bergeron *et al.*, 2002).

Following initiation of an immune response, the antigen-specific lymphocyte(s) proliferate and differentiate into effector cells that can eliminate the pathogen. A subset of these proliferating lymphocytes differentiates into memory cells capable of responding rapidly if the same pathogen entered again in future (Chang *et al.*, 2007).

Adaptive immunity can be divided into humoral (antibody-mediated) immunity (HMI), and cell-mediated immunity (CMI). Both types of immune response require activation of helper T cells (Th), an important lymphocyte that are essential for the development of effective adaptive immune responses. B-cells differentiate into plasma cells that produce antibody, or secreted immunoglobulin. Antibodies bind to extracellular pathogens or toxins, leading to their destruction. This is termed humoral immunity (Kuby, 2003).

Cell-mediated immunity provides protection against intracellular pathogens, transformed cells of the body, tumor cells and mutated body cell, e.g. antigen specific cytotoxic T-cells kill virally infected cells in an attempt to eliminate the virus. Another T-cell subset (helper T-cells) activates macrophages to kill microorganisms that reside within intracellular vesicles. Both mechanisms are included in the term cell-mediated immunity (Keogan *et al.*, 2006).

2.2 Cell Mediated Immunity (CMI)

Cell Mediated Immunity; CMI is an immune response that does not involve antibodies production, but rather involves the activation of phagocytes, natural killer cells (NK), antigen-specific cytotoxic T-lymphocytes, and the release of many cytokines in response to an antigen (Zabriskie, 2008). The targets for this type of response are cells rather than molecules, for example; because antibodies do not get into host cells, they are ineffective against intracellular pathogens; cell-mediated responses are the primary defense against these intracellular pathogens and the approach used is different depending upon where the pathogen resides in the host cell (i.e., in the cytosol or within vesicles). The viruses and some bacteria reside in the cytoplasm of the host cell, however, some intracellular bacteria and parasites actually live within endosomes in the infected host cell (Curiel *et al.*, 2003).

Cell mediated immunity responses, like most humoral immune responses, are tightly regulated and require help from Th cells, specifically the type 1 T helper cells (Th1, hence, the name Th1 responses), Th1 cells are characterized by their production of cytokines such as Interferon-gamma(IFN- γ), Tumor-necrosis factor-alpha(TNF- α), and Interleukin-2 that drive CMI responses. Both activated T helper cells and cytotoxic T-lymphocytes (CTLs) or TC play as effector cells in cell-mediated immune reactions. Cytokines secreted by T helper cells can activate phagocytic cells and enabling them to phagocytose and kill microorganisms more effectively (Delves,

2.2.1 Activation of CMI Cells

2011).

When T helper cells recognize foreign Ag on the surface of target cell in association with (or near) MHC class II; the TCR and cluster of differentiation CD4⁺ play role in recognition. Then Th cell will be activated and to produce cytokines (especially (IL-2 and IFN- γ)). These cytokines will activate TC CD8⁺ cells, MØ(macrophage) and NK cells. This activation will increase these cells ability for killing and became more effecter. After Th cells recognize specific antigen presented by an APC near MHC class II; they can initiate the key immune processes by selection; induction of proliferation of appropriate effector cells and enhancement of the functional activities of CMI cell (MØ, NK cells and TC) (Keogan *et al.*, 2006).

2.2.2 Importance of CMI Response

As classified by (Delves, 2011); the following represent CMI types:

- Defense against Tumor cells or cancer cells. Target cell is the cancer cells which have tumor markers.
- Grafts Rejection; an immune response against foreign cells or tissues that transplanted from other body even of the same species. Target cell is the foreign cells with foreign Ags.

- Defense against Mutation affected cells; defense against modified body cells or mutated cells with different surface antigens due to radiation or chemical agents' exposure. Target cell is the mutation cell with new Ags.
- Defense against Intracellular parasite infected cells; cellular immune response against infected cells with intracellular parasite (virus, bacteria, fungi and protozoa), with foreign Ag presented near MHC class I. Target cell is the infected cell with the intracellular parasite.
- Types –IV- hypersensitivity (Delayed type of hypersensitivity by the action of T_{DTH} a type of Th1cell and by cytotoxic T-cells as well as Th2 cells).

2.2.3 Mode of Action for Killing Target Cells

After T-cytotoxic cells and NK cells are activated by Th cells, T-cytotoxic cells come into close contact with target cell; they will bind to the Ag by their specific Ag receptors. While NK cells will attach to Ag (on Target cell surface) by one of their non-specific receptors for Ag. T-cytotoxic cells and NK cells will kill target cells by the following mechanisms (Walch *et al.*, 2014):

- a. Direct contact killing: Production of perforin, which represents a protein that is able to form pores in target cell membrane at the point of contact between Tc cell and target cell, lead to osmotic lysis of target cell(Voskoboinik *et al.*, 2015).
- b. Antibody-dependent cell-mediated cytotoxicity (ADCC) killing, it is specific killing and occurs when the parasites Ags have ability to induce both HMI and CMI, target cells will be coated with specific Abs formed after HMI against some parts of intracellular parasite like virus. These Abs will bring TC and NK cells very close to the target cell by acting like a bridge because TC and NK have receptors to the constant region of Ab. Then Tc and NK cells will be activated and kill the target cell by extracellular products (toxins and enzymes). This type of CMI usually occurs when the foreign Ag persist for long time (e.g *Mycobacterium tuberculosis* infection is long standing intracellular infection), also, against some kinds of cancer cells (Milligan *et al.*, 2015).

2.2.4 Neutrophils and the Immune System

Neutrophils are one of the PMNs cells, they represents the most abundant type of granulocytes in human blood. Hence they are the first arrivals to the site of infection. They migrate to start phagocytosis and eliminate invading pathogen by killing and lysing them. In addition, they contribute to tissue damage that occurs upon inflammation (Brinkmann *et al.*, 2004).

Neutrophils are an important soldiers of innate immunity, usually derived from stem cells in bone marrow with a lifespan maximum five days (Lewis, *et al.*, 2006).

After activation of these cells by an invasion of foreign pathogen and tissue damage, they will use their pseudopodia formation process to reach inflammation site

and generate phagocytosis as the first step of cellular innate defense of the body (Kolaczkowska & Kubes, 2013).

These cells number and activity were for long time representing an indicator for the clinical condition of human body, whereas, any change in their number increase or decrease will reflect a clinical case and their activity as well (Lewis *et al.*, 2011)

2.2.5 Cytokines as Communication and Regulation Molecules

In order to mount and coordinate an effective immune response, a mechanism by which lymphocytes, inflammatory cells and hematopoietic cells can communicate with each other is required; cytokines perform this function (Kuby, 2010).

Cytokines are a large, diverse family of small proteins or glycoproteins. The term cytokine is a general term used to describe a large group of proteins but there are other terms that are commonly used to describe particular kinds of cytokines. These include: 1) Monokines, cytokines produced by mononuclear phagocytic cells; 2) Lymphokines, cytokines produced by activated lymphocytes, especially Th cells; and 3) Interleukins, cytokines that act as mediators between leukocytes (Keogan *et al.*, 2006).

2.2.6 Cytokines Involved in CMI

Cytokines produced by Th1 cells activate macrophages and participate in the generation of TC cells, resulting in a cell-mediated immune response (Duque& Descoteaux, 2014)

2.2.6.1 Interleukin2 (IL-2)

Although interleukin-2 is produced by Th cells, it can also be produced by TC cells to a lesser extent. It is the major growth factor for T-cells. It also can activate NK cells and monocytes. It is a protein that regulates the activities of white blood cells (leukocytes, often lymphocytes) that are responsible for immunity. It is one of hematopoietic growth factors with a molecular weight 23 kD of 150 amino acids. key Interleukin-2 has roles in functions of the immune system, tolerance and immunity, primarily via its direct effects on T-cells. In the thymus, where **T**-cells mature. it prevents autoimmune diseases by promoting the differentiation of certain immature T-cells into regulatory T-cells, which suppress other T-cells that are otherwise primed to attack normal healthy cells in the body (Liao et al., 2013). Also IL-2 promotes the differentiation of T-cells into effector Tcells and into memory T-cells when the initial T-cell is also stimulated by an antigen, thus helping the body fight off infections. This cytokine was involved recently in the treatment of cancer (such as melanoma and renal cell cancer) and viral infected patients due to its role as biological therapy drug for enhancement of CMI response (Boyman & Sprent, 2012).

2.2.6.2 Interleukin 12 (IL-12)

Interleukin-12(IL-12) is produced by activated macrophages and dendritic cells. It stimulates the production of IFN- γ and induces the differentiation of Th cells to become Th1 cells. In addition, it enhances the cytolytic functions of TC and NK cells. It is produced by activated monocytes/macrophage, B lymphocytes, and connective tissue type mast cells as a 70 kD heterodimeric glycoprotein comprised of disulfide-bonded 35 kD and 40 kD subunits. Interleukin-12 is linked with autoimmunity. Administration of IL-12 to people suffering from autoimmune diseases was shown to worsen the autoimmune cases (Duque& Descoteaux, 2014).

2.2.6.3 Interleukin 18 (IL-18)

Interleukin-18 (IL-18) is an interferon gamma inducing factor, it is a recently characterized cytokine that shares structural features with the IL-1 family of proteins. Like IL-12, IL-18 it is produced by activated macrophages such as liver Kupffer cells and other resident macrophages, IL-18 is an early inducer of the Th1 response, co-stimulating the production of IFN- γ , IL-18 is expressed in several human diseases including rheumatoid arthritis and inflammatory bowel disease (Duque& Descoteaux, 2014).

Interleukin-18 (interferon-inducing factor) and IL-12 exhibit a marked synergism in interferon- γ induction in T cells. Investigations into the mechanism of this synergism have revealed that IL-12 up regulates expression of the IL-18 receptor on cells producing interferon- γ . Although IL-18 does not induce the development of Th1 cells, it is essential for the effective induction and activation of Th1 cells by IL-12 (Novick *et al.*, 2013). As for natural killer cells, IL-18 seems to activate them independently of IL-12. Although IL-12 and IL-18 activate both innate and acquired immunity, their excessive production by activated macrophages may induce multiple organ disorders including disruption of the immune system. This Interleukin has been used recently in cancer therapy (mainly for prostate cancer patients) for enhancement of better CMI (Keogan *et al.*, 2006).

2.3 Radiation

Radiation is the emission or transmission of energy in waves or particles through a space or material medium (UNSCEAR, 2010). The radiation has a lot of energy because it travels so fast. Radiation is made of particles which are smaller than atoms or waves and have no mass hence it is able to travel through objects that are solid to reach us (Hargreaves& Moridi, 2010).

Radiation is categorized as ionizing and non-ionizing, depending on the energy of radiated particles (Bessonov, 2006). Non-ionizing radiation refers to any type of electromagnetic radiation that does not carry enough photon energy to ionize atoms

or molecules that is to completely remove an electron from an atom or molecule such as Ultra violet (UV) in sunlight, visible light, microwaves, infrared and radio waves (Valko *et al* ., 2004).

Ionizing radiation (IR) is radiation with enough energy to remove electrons from atoms or molecules, causing ionizing the atom and then become charged, it has enough energy to produce free radicals, changing cross linkage between macromolecules, produce new chemical bonds and damage molecules in human cells that regulate vital cell processes like DNA and RNA which in turn may lead to cancer (Cohen, 2002).

The dose is delivered to tissue from ionizing radiation can either be acute (the energy from the radiation is absorbed over a few hours or days) or chronic (the energy is absorbed over a longer period of months, years, or over a lifetime) (Forster *et al.*, 2002).

2.3.1 Ionizing Radiation Types and Properties

2.3.1.1 Alpha radiation (α-ray)

This IR is charged particles, positive able to lose energy rapidly when pass through materials leading to ionization of 3molecules; cannot penetrate very far into materials and thin paper can shield them, e.g. α -ray particles emitted from Uranium ²³⁸U decay (McCmurry & Fay, 2008).

2.3.1.2 Beta radiation ($_{\beta}$ -ray)

This ray is particles also; they are electrons and are able to penetrate further than alpha particles. The Aluminum foil is able to shield them e.g. β –ray emitted from Thorium ²³⁴Th decay (McCmurry & Fay, 2008).

2.3.1.3 Gamma radiation (**y** –ray)

This radiation is low length waves with a very high frequency. This type of radiation is uncharged with high ability to penetrate deep in matters and ionize molecules of substances, e.g. X-ray. It can be shielded only by using lead plates or concrete (McCmurry & Fay, 2008).

2.3.1.4 Neutrons radiation

A very powerful radiation and similar to gamma radiation in all characteristics except they are particles, e.g. neutrons resulted from nuclear reaction of hydrogen nuclei (McCmurry & Fay, 2008).

2.3.2 Sources of Radiation Exposure

- **Natural background radiation** comes from cosmic rays from our solar system and radioactive elements normally present in the soil. This is the major contribution to worldwide radiation exposure (Hendry *et al.*, 2009).
- **Medical radiation** is used for medical therapeutic purpose like radioactive isotopes for cancer treatment, X-rays, CT scans, and other tests, as well as for radiation therapy (Mettler *et al.*, 2008).
- Non-medical, man-made radiation is used in small amounts in food irradiation, airport security scanners. Exposure to man-made radiation can happen in certain workplace, or in communities as a result of above ground nuclear weapons testing and nuclear accidents (Sherer *et al*., 2014).

2.4 X-radiation or X-ray

X-radiation is a form of electromagnetic radiation, X-rays have the shorter wavelength and therefore; more energy, than Ultraviolet radiation. They have a much shorter wavelength than visible light. Most X-rays have a wavelength in the range of (0.01 to 10) nanometers and energies in the range (100 ev); it is the amount of energy lost by the charge of a single electron moving across an electric potential difference of one volt to 100 kev) (Meo, 2004).

X-rays can pass through many solid materials. Accordingly, taking photographs with X-rays is used in medicine in order to see bones and abnormal things inside the body depending on three things Rayleigh scattering, Compton scattering and photo absorption. The images show bone because it is dense enough that X-rays are not able to pass through it, but for other parts of the body; normally; the X-rays are either absorbed or scattered (Kissel, 2000).

The absorbed X-rays by the body during medical radio-graphing procedures release energy to produce ionization. Ionization is the release of electrons from atoms and molecules which may cause chemical and biological change. The more X-rays absorbed during an exposure, the greater of possible biological change.

Although the ionization process occurs at the time of the exposure, any potentially harmful effects can take years to appear; an exposure to X-rays does not make a person radioactive nor lead to any residual radiation in the body to stay as a result of an X-rays exposure. X-rays can change living cells of human body upon high doses for a long time of exposure (Neumaier *et al.*, 2013).

2.5 Medical Use of X-rays

2.5.1 Radiography

It is very common in human lives to be exposed to this familiar process, usually during looking at broken bones or at the chest or teeth. A special big instrument directs a beam of X-rays through the part of our body that to be examined and on to a special film, then a photograph is produced on that film showing the structures that the X-rays have passed through in our body (Price & Le Masurier, 2007).

2.5.2 Fluoroscopy

Also it is called screening, when the X-ray beam is moving through human body; it will be viewed via a special camera which produces a moving photograph on a TV screen. The radiologist or radiographer during the examination can take snapshot of any important finding, or record the whole thing on video. Fluoroscopy examination is usually used to look at the gut and involves higher radiation doses than other radiography techniques (Schueler, 2000).

2.5.3 Computed Tomography Scan (CT)

This is a high professional way of using the X-ray source and a detector rotate inside the machine, the patient then lie on a narrow table which passes through a circular hole in the middle of that machine. A beam of X-rays passes through a slice of patient body on to a bank of detectors. An image will be formed by a computer and TV screen during slowly moving of patient through the hole to take different pictures of his body to produce 3D picture; the radiation dose in this technique is higher than fluoroscopy (Mac Manus *et al.*, 2003).

2.6 X-rays Technicians

Radiologist or radiographer is a person who, under supervision of a physician; operates radiologic apparatuses and equipment and assists other health professionals, he must be highly qualified with specialized education and training to enable him using equipment safely in all areas of the radiology department (Hall &Giaccia, 2006).

As working risk managements for radiation field workers, they undergo seasonal checkup tests for blood and film badges analysis (WHO, 2009).

Blood tests,(**Appendix A**), usually involves total and differential W.B.C.s count, Hb %, pcv% (Packed cell volume), and ESR (Erythrocyte Sedimentation Rate), while film badge analysis, **picture (2-1)**, include detection of the total seasonal exposure to

radiation for each worker. These film badges are packed with filters designed especially for recording radiation. These records are used to calculate the total dose of exposure for each person. These periodic checkups are made and watched in Iraq by the Ministry of Environment, Radiation protection center, Department of personal exposure monitoring. The calculated dose that obtained from film badges analysis do not represents the actual dose because sometimes the radiologist forget to put on them during work, leave them near the radio source instrument, or put them on under the gray lab coats. Time factor and work load can cause high dose of radiation exposure. For example; X-ray technicians, catheter doctors, expert doctors, dentists & service employees in the year 2006 had a percentage of 0.6% were recorded with overdose; while the percentage rose to 1% during the year 2008 (Al-Hamadany, 2011).



Picture (2-1): Film Badge used by Radiation Technician in Gynecology and Pediatric Teaching Hospital. Al-Muthanna Governorate.

2.7 Cabinet X-ray Machines

Cabinet of X-ray machines are highly enclosed, self-shielded, interlocked irradiation chambers. The machine cannot operate only when the chamber door is closed. The radiation exposure rates at every location and for uncontrolled areas (ICRP, 2013).

2.8 Film Badge

The film badge is one of the most commonly used personal monitoring device for X and gamma radiation and charged particles in Iraq (Ministry of Health and Environment, 2016). A film badge is composed of a piece of photographic film and a special film holder. The effect of radiation exposure is by darkening of the film, and the amount of darkening is proportional to the dose absorbed by the film. That film is placed inside a packet with a holder of various filters (e.g. lead, tin, aluminum and plastic). Radiation that passes through the filters will produce a density distribution on the film from which the energy range and type of the radiation can be determined, **picture (2-1)** (ICRP,2013).

2.9 Radiation Dose

Calculation of radiation dose is different according to the exposure type, either for ordinary people or for radiation occupational, whereas, it is reasonably that radiation occupationals dose limit is higher than normal people due to their exposure to radiation upon working in radiation field (WHO, 2007).

There are many ways to estimate radiation dose, even there are many types of radiation dose, like effective dose, absorbed dose and equivalent dose. Each one of these doses is used for telling and describe radiation exposure according to different factors (Sali *et al.*, 1996).

Absorbed dose is used to estimate the concentration of energy deposited in tissue after ionizing radiation exposure, gray (Gy or mGy) is the unit used; Gy: defined as one joul of energy deposited in one kilogram of body mass, the old unit was rad " radiation absorbed dose ", whereas; 1 Gy =100 rad (Mayo *et al.*, 2003).

Equivalent dose, mostly known as the biological dose because it assesses the biological health risks of ionizing radiation expected from an absorbed dose, meaning the damage impacts after exposure. Units are Sv (Sievert) and μ Sv and usually determined per year of exposure. This dose is the most common dose type used to determine exposure and it is usually limited by the organization, committees, commissions and United's that concerns with ionizing radiation including the WHO, NCRP (National Council on Radiation Protection and measurements) and ICRP (International Commission on Radiological Protection) (NCRP, 2016).

Effective dose, also uses μ Sv and Sv as units for estimation, but this dose take in account each organ or tissue sensitivity to ionizing radiation and the harm impact level of each organ and tissue upon exposure to an absorbed dose, different human body organs have different sensitivity to ionizing radiation, this dose is usually used for long-term exposure. Although the limits of radiation dose is very depended on affected body part, but the annual total equivalent dose should not exceed 1 μ Sv y⁻¹ for public people and 20 μ Sv y⁻¹ (over five years) for ionizing radiation occupationals as published by the ICRP (ICRP, 2013).

These doses depended on scientific knowledge and data recorded. It is still recommended by the ICRP to follow ALARA principle " As Low As Reasonable achievable ", it is continue the best way to avoid radiation damaging effects (Valentin, 2002).

Table (2-1) demonstrates the various doses that a person (as patient) would be exposed to during different single medical treatments or processes (Cho, 2005).

···· ()· - 1 ··· ································		
Medical Examination	Dose (µSv)	
Dental X-ray	0.01	
Mammography	0.04	
Chest X-ray	0.10	
Chest CT	7	
Abdominal CT	8	

Table (2-1): Equivalent Ionizing Radiation Dose

2.10 Classification of IR Effects on Human Body:

Ionizing radiation effects can be broadly divided into two categories as in (Prasad and Menon, 2005) :

2.10.1 Deterministic Effects:

Deterministic effects are based on tissue damage or (tissue reaction) to IR and related directly to the absorbed dose; the severity of these effects increases as the dose increase. Example of these effects are nausea, diarrhea, skin damage and sterility (Eisenberg *et al.*, 2011).

These effects have a threshold determined and limited by ICRP, once the threshold has been exceeded, the effect severity will increase with dose (Little *et al.*, 2009).

2.10.2 Stochastic Effects

Stochastic effect of ionizing radiation are chance effects with probability of the effect increasing with dose, but the severity of the effect is independent of the dose received .Stochastic effects are assumed to have no threshold. Primarily cancer risk , but also hereditary disorders are stochastic effect with a combined determent ~5 % Sv (ICRP, 2013).

Hereditary effects of radiation (gremline mutations induced by radiation that are transmitted to the offspring and may result in congenital anomalies or increased risk of common multifactorial disease. Stochastic effects occur when cells are not killed, but are modified. Some of the changes may persist in daughter cells. Examples of stochastic effects are cancer in the individuals who have been exposed to radiation if the transformation occurred in a somatic cell, and hereditary disease in descendants of individuals exposed when the transformation occurred in a germ cell (i.e. oocyte or sperm cells)(UNSCEAR, 2010).

2.11 Types of Ionizing Radiation Exposure

2.11.1 Acute IR Exposure

Refers to high dose of IR received in a short period of time. These effects are called radiation sickness, symptoms are gastrointestinal disorders (nausea, fatigue, vomiting, loss of appetite), bacterial infections, hemorrhaging, anemia, loss body fluids and electrolyte imbalance (Greenberger, 2009).

There are also other effects appear biologically delayed like cataracts, temporary sterility in both genders, cancer (different types) and genetic effects. Extremely high level of acute IR exposure can result in death within a few hours, days or weeks as occurred after the nuclear disaster of the earth quick of Fukushima /Japan (2011), and the old Chernobyl disaster of Nuclear power plant explosion occurred in Ukraine (1986) (Duran *et al.*, 2013 & Waselenko *et al.*, 2004).

2.11.2 Chronic IR Exposure

Represents the continuous or intermittent exposure to low levels of IR over a long period of time. These effects can be observed after a time following initial exposure. Symptoms include mainly cancers, precancerous lesions, benign tumors, cataracts, skin changes and congenital defects and malformation (Fazel *et al.*, 2009 and Hall and Giaccia, 2006).

2.12 General Health Impacts upon X-ray Exposure

The famous incident that happened between 1935 and 1954 in the UK, focused lights on the side effects of using X-ray in medicine and the health impacts of such sensitive therapy.when; 14000 patients were treated in UK using irradiation method to cure ankylosing spondylitis cases, a non-cancerous inflammation of spine; many of these patients had developed cancer post treatment with X-ray irradiation (Grdina *et al.*, 2002).

Either the patients or X-ray staff are in danger of X-ray radiation side effects, X-ray technicians are in danger of receiving unnecessary radiation dose from both primary beam and scattered radiation specially upon bad managements and with no protective devices (Williams & Fletcher, 2010).

Over dose of X-ray can effect human body in general; either on systemic level or cellular level. According to systemic level; gastrointestinal tract is the first responser against IR, at doses above 7 Gy (700 rad), injury to the gastrointestinal tract contributes increasingly to the severity of the manifest-illness phase. Such high exposures inhibit the renewal of the cells lining the digestive tract. These cells are short lived and must be renewed at a high rate. High exposures then lead to depletion of these cells within a few days. Thus, the result of high exposures is a breakdown of the mucosal lining and ulceration of the intestine (Greenberger, 2009). As the mucosa breaks down, bacteria can enter the bloodstream and are unchallenged because of the curtailed production of granulocytes. These conditions develop over a few days and are characterized by cramping, abdominal pain, and diarrhea, followed by shock and death (Kirsch *et al.*, 2010).

Hematopoietic system (including the immune component) is the most targeted part of human body by IR including X-ray. The hematopoietic stem cells are the most radiosensitive tissues in the body. Radiation doses of 2 Gy (200 rad) or more can significantly damage the blood forming capability of the body. Acute doses kill some

of the mitotically active precursor stem cells, diminishing the subsequent supply of mature red cells, white cells, and platelets (Greenberger, 2009).

As mature circulating cells die and the supply of new cells is inadequate to replace them. The physiological consequences of hematopoietic system damage become manifest (Nana *et al.*, 2012). The damage to bone marrow leads to symptoms such as increased susceptibility to infection, bleeding, anemia, and lowered immunity. There may be fever and rises in pulse and respiratory rates due to endogenous bacterial and mycotic infections. If at least 10% of the hematopoietic stem cells remain uninjured, recovery is possible. Otherwise, death occurs within 4 - 6 weeks (Hall & Giaccia, 2006).

Other general effects like integumentary system damage including hair loss and skin maculation; moreover different skin cancers like melanoma (Morgan & Sowa, 2015).

Reproductive system for both genders can be affected by over X-ray leading to infertility, abnormal forms of sperms, abortions, still births and congenital malformations (Mettler, 2012).

On the cellular level; if X-ray strikes the body, it randomly hits millions of cells, radiation may simply passes through some of them with no harm done. Other cells mainly whom hitted by IR directly may be killed or damaged. If the resulted damage is able to be repaired then the cell will survive and repair itself with no permanent damage. But; if the damage is no repairable; so the cell shall die like millions of normal cells do naturally in human body by apoptosis process. The dead cell debris is carried away by the blood and a new cell is usually generated through normal biological processes to replace it (Paglin, 2001).

The most critical cellular damage occurs when the cell remain alive but with a mutation. Whereas this cell will exhibit a change in the cell's reproductive structure and loss self-control system allowing to generate as a potentially pre-cancerous cell leading to malignancy and cancer formation (Greenberger, 2009).

Inside the cells, at the biochemical and molecular levels; when X-ray radiation is absorbed in the cell, it is possible that the radiation interacts directly with critical elements in the cell. The atoms of the target may be ionized or excited, initiating a chain of events which lead to biological change. Therefore, IR pass through cells will ionize molecules making them unstable. Some chemical bonds will break while others will form (unfavorable bonds) causing loss of the biological function of those molecules. In this situation, damage may be transmitted to succeeding generations of cells, making the damage in this instance cumulative with radiation dose (Akleyev, 2014).

For example, enzymes are proteins which represent very important macromolecules inside living cells. When ionizing radiation breaks their functional form bonds (folding bonds) forming unfunctional enzyme protein that will block the reactions that enzyme is involved; resulting stop of these reactions products supply.

Some references call this effect as direct chemical effects, (Pala& Tabakçioglu, 2007).

Indirect chemical effects represent water molecules ionization .Absorption of radiation energy may produce a chemical reaction called free-radical formation. A free radical is a free atom or molecule carrying an unpaired orbital electron in the outer shell. An atom with an unpaired electron in the outer shell usually exhibits a high degree of chemical reactivity. The two substances in a cell likely to be involved in free radical formation due to ionization are oxygen and water since living cells are composed mostly of water which when ionized will form highly reactive free radicals (O2-, OH° ,H2O+, and O°) and the most strong oxidizing agent (H2O2) Hydrogen Peroxide; see equations (2-1) and (2-2). These radicals will destroy the internal biochemical systems and biomolecules of life causing cell death as a final fate (Tseng *et al.*, 2003).

 $2 H_2O \xrightarrow{\text{Radiation}} H_2O_2 + 2H^{\circ} \qquad (2-1)$ $H_2O_2 + 2H^{\circ} \xrightarrow{\text{OH}^{\circ}} + H_2O^{\circ} - (2-2)$

While the biological effects of ionizing radiation are the combined result of direct absorption of energy at molecular level and the indirect oxidative damage produced by the reactive oxygen species (free radicals) produced through a process called water radiolysis. Direct effect may lead to recognizable damage particularly when they effect molecules of biological importance. The DNA molecule is principal target for the biological effect of ionizing radiation , including cell killing and mutation leading to non-lethal cell transformation (UNSCEAR, 2010).

For low levels of exposure, the biological effects are so small that they may not be detected. The body is able to repair damage from radiation, chemicals and other hazards. High radiation doses (again, greater than 50,000 mrem, or 500 μ Sv) tend to kill living cells. Low doses may damage or alter a cell's genetic code (DNA). High doses can kill so many cells that tissues and organs are damaged immediately (Greenberger, 2009).

On the genetic level; during the past two decades scientists focused on molecular mechanisms of damage in human cells affected with ionizing radiation due to the improvements of tissue culture techniques which gave hand to many researches in order to understand the carcinogenesis induced by IR. X-ray radiation can lead to molecular changes and damage chromosome material. This damage takes the form of changes in the construction and function of the cell (Prasad and Menon, 2005).

X-ray is able to induce mitochondrial damage through the alteration of mitochondrial permeability transition, increase the accumulation of reactive oxygen species, and the decrease in ATP production (Morgan, 2003).

When DNA and RNA, macromolecules of life, are ionized the reproduction of new cells will be affected. If the cells were somatic cells, then damage can prevent

the cells from dividing and thus stop repairing damaged tissues. In addition, DNA mutation can result in uncontrolled division of these cells leading to cancers formation. If reproductive cells (ova and sperm) are damaged this will lead to abnormalities in offspring, deformities fetuses, still births, abortion, and other serious problems like infertility (Azzam, 2003 and Lorimore *et al.*, 2001).

Ionizing radiation plays a role in activation of oncogenes (cancer genes found normally in cells genome) or tumor suppressor genes (control genes in cells genome) and loss of cell-cycle check points (Dent, 2003); leading to increase in mutations frequency and changing gene expression in neighboring cells that received no direct radiation exposure (Baldwin, 2001). The cells of direct exposure mostly will suffer from early apoptosis (a regulated process of programmed cell death involving a cascade specific cellular events leading to the death and lysis of that cell) (Dent, 2003).

2.13 The Immune System and X-ray

X-ray affects and interfere with the immune system via cellular level, IR exposure is cumulative process matter, when the person gets IR more than the threshold dose. All body cells will be affected including the immune cells. Moreover the cells that die and the immune system will lose their efforts; there will be unbalance and disregulation of the immune system steady state (Safwat, 2002).

Many scientists suggested that free radicals that are formed after X-ray exposure are able to bind to and destroy cytokines, also these free radicals mutate DNA of affected immune cells leading to change cytokines receptors sites on their surfaces hence losing communication and regulation of the immune system and then disturbance of general function (Prasad and Menon, 2005).

Many scientists investigated the relation between IR and immune system health basing on the survivors of Hiroshima & Nagasaki bombing, Chernobyl resident people, Fukushima power plant emergency workers and patients undergoing radiotherapy. Most data showed that high-dose of irradiation can damage the immune system. However, some recent studies reported that low dose for little time have a modulating effects rather than bad impacts on immune system hence using radiotherapy of cancerous cells (Greenberger, 2009).

Human health, environment, diet, age, physiological stress, race smoking and other factors that influence the immune system can increase the persons sensitivity to IR (Heidrich *et al.*, 2007).

Real damage of the immune system usually follows moderate to high-dose of exposure to IR. The lymphoid cells will be affected hence antibodies and cytokines production will be affected too (Greenberger, 2009).

Many scientists suggested that neutrophils, the most important component of the cellular immunity is the first cell that responds to radiation harm either by number or function (Al-Hamadany, 2014).

When the stem cells are damaged; the precursors of all hematopoietic cells; then the resulting new cells that formed will be abnormal in number or/and function (Greenberger, 2009).

From another point, suppressing lymphoid cells upon overdose of irradiation and disregulation of the immune system can participate in developing cancers, since CMI is the real bodyguard that keeps human body from cancerous cells when they were detected by Th cells as target cells for CMI. Developing cancer upon overdose of irradiation may belong to loss CMI rather than mutated cell due to genetic disorder (UNSCEAR, 2010).

Effect of IR on healthy individuals depends on the total dose and dose rate of radiation exposure . High dose IR , given acutely at high dose rate, is generally considered to be detrimental, causing apoptosis, DNA damage, and transformation of cells into tumor cells (Hall, 2000; Ross, 1999 and Aoyama *et al.*, 1998).

In addition to the direct effect of radiation, focal radiation can have distant effects that influence tumor growth outside the irradiated region, and also it has the potential to damage the DNA within our body's cells, and if DNA molecules is damaged, the effect could be that the cell reproduces often and before it reaches a mature state or being functional. The new cells will also have the same defect and behave in the same way. The result is a growth of cells with no beneficial function in the body. This is a tumor, if the tumor invades other tissues in the body, it is called cancer (Little, 2009).

Cells of the immune system, like most radiosensitive tumors, can also be rapidly dividing, and are vulnerable to radiation. Radiation exposure induces apoptosis in mature NK cells as well as T and B lymphocytes and lethal damage in bone marrow stem cell precursors of monocytes and granulocytes. In individuals receiving heavy doses of radiation, for example, atomic bomb survivors, both mature lymphocytes and bone marrow stem cells were severely damaged, causing profound depletion of granulocytes and natural killer cells (Eriksson and Stigbrand, 2010).

2.14 Prevention X-ray IR Effects

There were critical limits for radiation exposure of an individual laid down by (ICRP), World Nuclear Organization (WNO), and Nuclear Regulatory Commission (NRC) to ensure that radiation dose received by any person (other than an accidental exposure, or a deliberate exposure in medical diagnosis), does comply with the followings: (Seaton, 2004).

- The probability of any effect of the stochastic type is small enough to be acceptable to the individual and society.
- The dose is below the threshold for any biological effects (nonstochastic or deterministic).

Many countries take the ICRP values for radiation exposure as reference, where one μ Sv/ year represents non-radiation workers and public dose (should be equal to

or less than), while for radiation occupational workers the dose is 100 μ Sv/5 years or 20 μ Sv/ year (ICRP, 2013).

It is essential that radiation workers be protected when they need to work outside the protective cubical. There are several essential protective devices including protective clothing which should be readily available for use in every X- ray room. These devices are used to protect staff from receiving unnecessary radiation dose from both the primary beam and from scattered radiation . These devices should also be used to shield members of the public from unnecessary dose for example when a parent holds the arms of baby during exposure of a chest radiograph . The ALARA principle should be applied for every exposure made to patients (WHO, 2009).

Avoid waiting around X-ray rooms to avoid unnecessary dose of X-ray especially by pregnant women, X-ray technicians must follow instruction of safety and do the periodic checkup as recommended by the WHO. Also, X-ray room should always be checked and examined by specialists to ensure proper work and high levels of safety (ICRP, 2013 and WHO, 2007).
CHAPTER THREE MATERIALS AND METHODS

3.1 Materials

3.1.1 Subject

A total of (60) volunteers were included in this study, they were all X- rays technicians working in Al-Muthanna governorate hospitals. Those workers were healthy people since they do not suffer any clinical sign. Cases included adult males and females. Samples were taken from women not during menstruation period (taken during the first 10 days of menstrual cycle). Cases ages ranged between (21 and 60) years.

3.1.2 Controls

A total of (29) volunteers were healthy adult people aged (23 and 45) years) of both genders. They were with no clinical sign for any disease and with no history of working with X- rays or any radioactive material before. Menstruation period was avoided in women control involved in this study.

3.1.3 Samples

Five milliliters (5 mL.) of blood samples were taken by venipuncture from all cases and controls involved in present study. Each blood sample was divided immediately into two portions; the first one (1.5mL) was put in heparinized tube and gently left to mix; and the second portion (3.5 mL) was put in non-heparinized tubes and left in the incubator at 37°C for (30-45min) for clotting, then serum was separated by centrifugation for (15 min at 3000 round per minute r.p.m) (Lewis *et al.*, 2006).

No hemolysed serum included and separation was accomplished under septic conditions and protected from evaporation then serum samples were divided into two parts in Eppendorf tubes after labeling them, and stored at -20°C in the freezer (Mckenzie, 2004). Samples collection started in (December) 2015 and finished in (March) during 2016.

3-1-4 Cases Grouping

This research included X-ray technician and controls to be tested immunologically. Controls were (29) persons used as a group for comparing during statistical analysis for all results and outcomes in this study.

Moreover; for statistical reasons and as recommended by (ICRP,2013); X-ray technicians were divided into two groups. Whereas, G1 contained samples of technicians whom employment years were less (and/or) equal to (5) years, and contain (23) persons, and G2 contained cases of occupationals of more than (5) years employment in radiation field, and contain (37) persons.

Also; the results of the two parameters being used in this study (Total W.B.Cs count and Hemoglobin percentage) were treated statistically post separation the two genders, whereas; the statistical comparison of each gender result was done with the same gender outcomes of controls; as recommended by (Meckenzie, 2004).

3.1.5 Questionnaires Form

Data about all cases involved in this study and related to our research are summarized in appendix B.

3.1.6 Chemicals

The chemical which are used in the present study are listed in table (3-1)

Table (3-1): The chemicals used in the present study

No.	Chemical	Source
1	Methyl alcohol	Merk (Germany)
2	Glacial acetic acid	BDH (UK)
3	Dihydrogen potassium phosphate	BDH (England)
4	Phosphate Buffered Saline (PBS)	BDH comp. (UK)

3.1.7 Stains

The stain which are used in the present study are lited in table (3-2).

 Table (3-2):
 The stains used in the present study

No.	Stains	Source
1	Leishman's Stain	Fluka (Germany)
2	Nitrotetrazolium Blue Chloride	Sigma (USA)
3	Gentian Violet	Fluka (Germany)

3.1.8 Kits

The kits which are used in the present study are listed in table (3-3)

Table (3-3): The kits used in the present study

No.	Kits	Source
1	EIA IL-2 (ELISA)	Biolegend (USA)
2	EIA IL-12 (ELISA)	Biolegend (USA)
3	EIA IL-18 (ELISA)	Elabscience (China)

3.1.9 Laboratory Apparatuses and Equipments

Equipments and apparatuses used in this study are listed in the table (3-4).

Table (3-4): Equipments and Apparatus

No.	Apparatus and Equipment	Source
1	Autoclave	Gallenkamp (England)
2	Bioelisa Reader	BioTek (USA)
3	Bioelisa Shaker	TKA 226-100S (Italy)

Bioelisa Washer	BioTek (USA)
Centrifuge	Hitachi (Japan)
Digital Camera	LG (Korea)
Digital Hemolyser	Sysmex (Japan)
Disposable Syringe 5 mL	Samaraa (Iraq)
Eppendorf Tubes	Bioneer (Korea)
Gloves	China
Heamocytometer	Neubauer (Germany)
Heparin Tubes	Jordan
Ice box	China
Incubator	Yamata (Japan)
Light microscope	Olympus (Japan)
Micropipette	Dragon MED (Koria)
Micropipette multichannel	Dragon MED (Koria)
Micropipettes 1-100, 100-1000Ml	Korea
Neubauer Haemocytometer Chamber	Germany
Nano Drop	Optizen (USA)
Oil (for oil immersion)	China
Plain Tubes	DMD-DISPO (Syria)
Refrigerator	Concord (Lebanon)
Sterile Test Tube	Superestar (India)
Tips	China
Turnica	Korea
Vortex	Karlko18 (China)
	Bioelisa WasherCentrifugeDigital CameraDigital HemolyserDisposable Syringe 5 mLEppendorf TubesGlovesHeamocytometerHeparin TubesIce boxIncubatorLight microscopeMicropipetteMicropipettes 1-100, 100-1000MlNeubauer Haemocytometer ChamberNano DropOil (for oil immersion)Plain TubesRefrigeratorSterile Test TubeTipsTurnicaVortex

3.1.10 Solution Preparation

3.1.10.1 Leishman's Stain

This stain was prepared by dissolving the powdered Leishman's stain (0.2 gm) in (100 mL) of methyl alcohol, all the stain was dissolved, and the formed stain solution was filtered and kept in a dark glass bottle inside the incubator at 37° C, till usage (Lewis *et al.*, 2006).

3.1.10.2 Preparation of Buffer Solution for Leishman Staining

This buffer was prepared by mixing of two prepared solutions , solution No. I and solution No. II (Lewis *et al.*,2011)

-Solution No .I: prepared by dissolving (8 gm.) of Sodium Hydroxide in 1000 mL. of Distilled water D.W.

-Solution No. II: dissolving (72.2 gm.) of Potassium Dihydrogen phosphate in 100 mL. of D.W.

3.1.10.3 Preparation of Diluting Fluid for Total WBCs Count:

This fluid was prepared by mixing of the following materials (Lewis et al., 2011)

No.	Material	Volume
1	Aqueous solution of Gentian Violet 1%	1.5 mL
2	Glacial Acetic acid	1 mL
3	Distilled water	98 mL

3.1.10.4 Phosphate Buffered Saline (PBS)

This buffer solution was prepared as in (Lewis *et al.*, 2006), by dissolving (9 gm) of ready powdered PBS into (1000 mL) of D.W, the buffered solution (pH=7.2) was sterilized using autoclave and kept in refrigerator until usage.

3.2 Methods

3.2.1 Design of the Study



Figure 3.1: The study design of present work

3.2.2 Haematological Methods

3.2.2.1 Total W.B.Cs Count

Whole blood was diluted with W.B.Cs diluting fluid and the neubauer haemocytometer chamber was used to count the W.B.Cs. Sample application was done after colour changed upon time end of test as in (Lewis *et al.*,2011) to ensure breaking of R.B.Cs in blood sample.

The total number was calculated by using the following equation :

Total W. B. Cs =Cells counted in 4 squares/4 \times dilution factor \times 10 (Cell/mL)......(3-1)

3.2.2.2 Differential W .B.Cs Count

After preparation of the thin blood films, the smears were left to dry then stained by Leishmans' stain for 5 min., buffer solution was used to dilute the stain that covering the smears slides to keep pH original while staining and avoiding change in blood cells morphology. After drying of slide, they were examined under oil emersion by counting (100) white cells and identification of their types, then percentage of each type of white cells was calculate of (Lewis *et al.*,2011).

3.2.2.3 Hemoglobin Measurement

Hemoglobin was measured by automatic machines as routinely done for blood sample in Al-Muthnna Governorate hospitals, the Hematology Laboratories technicians usually use digital hemolyser (Sysmex). Within the machine, the red blood cells are broken down to get the hemoglobin into solution. The free hemoglobin is exposed to a chemical containing cyanide that binds tightly with the hemoglobin molecule to form cyanomethemoglobin. By shining a light through the solution and measuring how much light is absorbed (specifically at a wavelength of 540 nm). The amounts of hemoglobin were determined (Theml *et al.*, 2004).

3.2.3 Immunological Methods

3.2.3.1 Principle of Nitro-blue Tetrazolium (NBT) Test

This test is one of the standard neutrophils function tests. It involves detection of respiratory oxidative burst activity in neutrophils via their ability to reduce NBT dye after mixing the dye with heparinized blood in equal volumes (Edgar, 2006).

Normally, all phagocytic cells are able to engulf the dye by phagocytosis process, reducing and depositing it as dark blue crystals which are named (Formazan particles). The cells that have the appearance of a single large black deposit intracellularly; (or multiple black speckles randomly distributed in the cell cytoplasm (inside phagocytic vacuole or phagosome) were counted as positive, otherwise they were counted as negative) (Male, 2004).

This test was used to evaluate neutrophils phagocytic activity. Monocytes are also able to reduce this dye to formazen particles and produce a speckled appearance, hence they were differentiated and not included as positive cells during counting of films;, as well as eosinophils and basophils. Furthermore, some extracellular masses of formazan deposits may appear in slides smears. They were cancelled (Freeman and King, 1972).

3.2.3.2 NBT Assay Procedure

1. Preparation of the dye solution was according to (Al-Hamadany, 2011) by dissolving (1mg) of NBT powder in (1mL.) of PBS (pH=7.2), the light yellow solution obtained was centrifuged and supernatant was used directly to accomplish the tests. This solution was freshly prepared for each batch of tests. This step was made carefully since this dye know as highly toxic and its powder is volatile.

2. Heparinized blood samples were used to perform this test by mixing (0.1mL.) of heparinized blood with (0.1mL.) of NBT dye solution in Eppendorf tubes (equal volumes of blood and dye solution), the mixtures were incubated for 30 min at 37° C as recommended by (Freeman and King, 1972).

3. After incubation, thick blood smears on clean slides were prepared directly from these mixtures and air dried for the next step of staining.

4. Staining of prepared smears slides is done using the same procedure of blood film staining in differential W.B.Cs count using Leishman's stain (Lewis *et al.*, 2011).

5. Microscopic examination of all slides was done under light microscope using oil immersion, whereas 100 cells of neutrophils were randomly selected and identified whether they were positive or negative for NBT dye reduction (concerning the presence of Formazan particles precipitated) (Al-Hamadany, 2011 and Freeman & King, 1972).

3.2.3.3 Human Interleukin (IL-2) Kit

This kit of Enzyme Immune Assay (EIA) was specifically designed for the accurate quantitation of human IL-2 from serum and other biological fluids, ELISA technique was Sandwich Enzyme-Linked Immunosorbent assay (ELISE) with a 96-well strip plate that is precoated with a capture antibody (direct method). (The kit enclosed instruction leaflet).

The reagents that provided by this kit were:

- Anti-human IL-2 pre-coated 96-well strip micro plate
- Human IL-2 detection antibody
- HumanIL-2 standard
- Avidin-HRP A
- Assay buffer B
- Wash buffer (20X)
- Substrate solution F
- Stop solution
- Plate sealers

3.2.3.4 Human IL-2 Assay Procedure

1. Kit components and serum samples wre left for 30 min at room temperature before use, all serum samples were stirred using touch-mixer.

2. Wash solution was diluted using D.W as labeled on vials.

3.Serial dilutions were prepared as in kit leaflet instructions; these dilutions were prepared before use directly as recommend by manufacturing company leaflet.

4. A special map was filled before start working.

5. Addition of 50 μ L. of Assay buffer B to each well that contain either standard dilutions or samples then washing 4 times with wash solution.

6. Step 1: included application of 50 μ L. of diluted standards and serum samples to each well using multichannel micropipette according to the map. The microplate was then (after covering with closure membrane) incubated 2 hr. at 18-25°C (Room Temperature RT) while shacking vigorously (350 rpm) using microtiter plate shaker.

7. After incubation, the plate was wash by four-cycle plate washing mode using a microtiter plate washer and wash solution that prepared. This solution was completely aspirated when four cycles ended.

8. Step 2: included application of 100 μ L. of Human IL-2 detection antibody solution to each well, the plate was sealed and incubated at RT for 1 hour while shaking.

9. Washing the plate as in step 7 was done.

10. Step 3: included application of 100 μ L. of avidin-HRP A solution was added to each well, the plate was sealed and incubated at RT for 30 min while shaking.

11. Washing 5 times as in step 7 was done.

12. Step 4: a total of 100 μ L. of Substrate solution addition to each well and incubated for 20 min in the dark. Wells containing human IL-2 should turn to blue in color with an intensity proportional to its concentration.

13. To stop the reaction, total of 100 μ L. of stop solution was added to each well. The solution color was changed from blue to yellow.

14. The absorbance was read at 450 nm within 20 min upon colour change.

15. Calculations were done using standard curve and the corresponding IL-2 concentration were estimated according to the sample optical density OD value. A special program (Appendix C) provided by Biolegend Company was used to view results of samples (Kit enclosed instruction leaflet). Concentrations were obtained in (pg/mL.).

3.2.3.5 Human Interleukin (IL-12) Kit

This ELISA kit is a sandwish enzyme linked immunosorbent assay with 96 well strip plate that is pre-coated with captured antibody .This kit is specifically designed for the accurate quantitation of human IL-12(p70) for serum samples and other biological fluids.(The kit enclosed instruction leaflet).

The reagents that were provided by this kit were:

- Anti-human IL-12 (p70) pre-coated 96-well strip micro plate
- Human IL-12 (p70) detection antibody
- Human IL-12(p70) standard
- Matrix A

- Avdin-HRP A
- Assay buffer A
- Wash buffer (20X)
- Substrate solution F
- Stop solution
- Plate sealer

3.2.3.5 Human IL-12 Assay Procedure

1. Kit components and serum samples were left for 30 min at room temperature before use, all serum samples were stirred using touch-mixer.

2. Wash solution was diluted using distilled water (D.W) as labeled on vials.

3. Lyophilized human IL-12 (p70) standard was reconstituted with assay buffer A as stated on the vial label then solubilization done after 15 min since dispensing by mix gently to avoid foaming. This step obtained standard stock solution of IL-12 (P70) in 20 pg/ml.

4. A special map was filled before starting work.

5. Serial dilutions were prepared as in kit leaflet instructions; these dilutions can not be stored and were prepared before use directly.

6. Step 1: application of 50 μ L. of standard and serum samples to each well was accomplished using multichannel micropipette according to the map. The microplate was then (after covering with sealer) incubated 2 hr. at RT while shacking vigorously (350 rpm) using microtiter plate shaker.

7. After incubation, the plate was washed by four-cycle plate washing mode using a microtiter plate washer and wash solution that prepared. This solution was completely aspirated when four cycles ended.

8. Step2: a total of 100 μ L. of human IL-12 detection antibody solution is added to each well, plate was sealed and incubated at RT fot 1 hour while shaking.

9. Washing for 4 times; as in step 7; was done.

10. Addition of 100 μ L. of avidin-HRP A solution to each well and plate was incubated at RT for 30 min while shaking.

11. Washing step for 5 times with wash buffer was done.

12. Addition of 100 μ L. of substrate solution F and incubation for 20 min in the dark, wells containing human IL-12 were turned to blue in color.

13. Reaction was stopped by adding 100 μL of stop solution, the solution color was changed from blue to yellow.

14. The absorbance was read at 450 nm within 20 min.

15. Calculations were done using standard curve (liner regression equation) and finding the corresponding density according to the sample OD value multiplied by the dilution multiple. A special program provided by Biolegend Company was used to view results of samples actual density. (Kit enclosed instruction leaflet). Results were obtained in (pg/mL).

3.2.3.7 Human Interleukin (IL-18) Kit

This ELISA kit used sandwich-ELISA as method. The micro ELISA microplate provided in this kit was pre-coated with an antibody specific to IL-18. This cytokine molecular weight is (24) kD.

The reagents that were provided by this kit were:

- Micro ELISA plate 8 wells × 12 strip
- Reference standard
- Reference standard & sample diluent
- Concentrated biotinylated detection antibody
- Biotinylated detection Ab diluent
- Concentrated HRP conjugate
- HRP conjugate diluent
- Concentrated wash buffer (25×)
- Substrate reagent
- Stop solution
- Plate sealer
- Manua

3.2.3.8 Human IL-18 Assay Procedure

1. All reagents and samples were bringed to room temperature before use.

2. Serial dilutions were prepared as in kit leaflet instructions.

3. A total of 100 μ L of standards was added to each standard well, while 100 μ L of diluent was added to blank well and 100 μ L of sample was added to each sample well. Then microplate was covered with sealer and incubated for 90 min at 37 c.

4. Addition of 100 μ L. of biotinylated detection Ab were added then the plate was covered with the plate sealer and incubated for 1 hr at 37°c. (in the incubator).

5. Each well was aspirated and washed, the process is repeated three times using washing buffer.

6. A total of 100 μ L of HRP conjugate was added for each well and covered with sealer and incubated for 30 min at 37°c.

7. A total of 90 μ L. of substrate was added and a new plate sealer was used to cover the plate, then incubated for 15 min at 37°c then blue color appeared.

8. A total of 50 μ L. of stop solution was added to each wells, the color is turned from blue to yellow immediately.

9. Absorbance was read using micro spectrophotometer at wavelength of 450 nm.

10. Calculations were done using standard curve (liner regression equation) and finding the corresponding density according to the sample OD value multiplied by the dilution multiple. A special program provided by Elabscience Company was used to view results of samples actual density. Results were obtained in (pg/mL). (Kit enclosed instruction leaflet).

3.3 Statistical Analysis

All the obtained results for all members in this study were statistically analyzed using mean calculations (M) and standard Error (SE) using Statistical Analysis System SAS 9.1. ANOVA analysis with one direction variation was depended. Both groups G1 and G2 results were compared with the controls' results using student t-test to find the significance of increase or decrease for all the parameters, also G1

results were compared with G2 result for the same reason. The level ($p \le 0.05$) was used as the significancy level (SAS, 2010).

CHAPTER FOUR RESULT

CHAPTER 4: RESULT

4.1 Data Analysis

The present study project included data collection about X-ray technicians occupied in Al-Muthanna Hospitals during the year 2015-2016. A total of (89) cases were included in this study; their ages ranged between (21-60) years with a mean (35.9 years). There were (60) adults males and females as test cases of X-ray technicians from the total (89). While there were (29) adults as a control group randomly chosen from Iraqi culture in Al-Muthanna Governorate. Control ages ranged between (23-45) years with a mean (33.5) years.

Table (4-1) shows the distribution of X- ray technicians and control according to their total cases number, ages, gender and other data which were collected during this research.

Table (4-2) shows X-ray technicians distribution according to their occupation.

No.	Occupation Place	lace Number	
1	X-ray Photographer	32	53.3
2	CT Scan Room Technician	10	16.7
3	Screen Technician	11	18.3
4 Dentists		6	10
5 Mammographic Technician		1	1.7
Total		60 (%)	

Table (4-2) : X-ray Technicians Distribution According to their Occupation

The most obvious observation recorded during this study was that most of technicians involved did not have the periodic checkup for more than 18 months, moreover that; (36) technicians (60 %)from the total (60), have no license (film badge) to work legally in radiation field as X-ray technician as recommended by the Ministry of Health and Environment in Iraq.

Another observationwas tha, although has been recorded during samples collection; that there no clinical signs appeared on all technicians involved in this study, there were some indicators of radiation exposure consequences like hearing impairment, general body weakness, hair falling and pale faces in both technician genders.

4.2 Hematological Parameters

4.2.1 Total W.B.Cs Count

Normal range for this parameter is $(4-11) \times 10^3$ as (Lewis *et al.*,2011). Results for technicians did not exceed 10.8×10^3 , while the lowest recorded value was 2×10^3 (male). There were 15 cases (25%) from total 60 showed decreased levels lower than normal value. All control results were within normal range.

Table (4-3) and (4-4) shows statistical analysis for technicians results compared with controls according to gender, whereas; table (4-3) represents females' and (4-4) represents males' results.

 Table (4-1): Total X-ray Technician Cases and all Data Collected during the Study.

No.	Hospitals of	Total	Male	Female	Employment	Licensed	Periodically	Non-governmental
	Occupation	Cases No.	No. (%)	No. (%)	Years Range	No. (%)	Checkup	Clinic
		Collected						Employment
1	Al-Hussain	41	35(58.3)	6(10)	(1-42)	19(31.7)	25(41.7)	13(21.7)
	Educational Hospital							
2	Gynaecology and	3	3(5)		(10-42)	2(3.3)	2(3.3)	1(1.7)
	Pediatric Hospital							
3	Al-Rumaythah	5	4(6.7)	1(1.7)	(1-29)	2(3.3)	1(1.7)	1(1.7)
	Hospital							
4	Al-Khdir Hospital	4	3(5)	1(1.7)	(1-29)	1(1.7)	1(1.7)	1(1.7)
5	Specialized Dentology	7	3(5)	4(6.6)	(1-24)	0(0)	0(0)	1(1.7)
	Center							
		60	48(80)	12(20)		24(40)	29(48.4)	17(28.5)
	Total							

CHAPTER 4: RESULT

Statistical analysis illustrated that there were no significant differences obtained between G1 and G2 values with controls despite of that there were 25% values among cases under test recorded a decrease but it was insignificant in both genders.

No.	Group	Mean (10 ³)±SE
1	Control	8.48 <u>±</u> 0.08
2	G1	8.61 <u>±</u> 0.10
3	G2	8.49 <u>±</u> 0.16

 Table (4-3): Total W.B.Cs Count for Females (Cases and Control)

 $(M \pm SE)$: Mean \pm Standard error: mean $\times 10^3$

Table (4-4): Total W.B.Cs Count for Males (Cases and Controls)

No.	Group	$Mean(10^3) \pm SE$
1	Control	8.38 <u>+</u> 0.05
2	G1	8.59 <u>±</u> 0.06
3	G2	8.46± 0.08

 $(M \pm SE)$: Mean \pm Standard error :mean $\times 10^3$

4.2.2 Differential W.B.Cs Count

4.2.2.1 Neutrophil Counts

In general, neutrophils percentages obtained for all technicians involved in this study ranged between (33-80%) with a mean value (54.3%). There was no elevated value recorded. All control percentages for neutrophils were normal and within accepted values. Normal range depended was (40-80%) (Lewis *et al.*, 2011).

Table (4-5) shows that neutrophils counts results, were significantly different at the level of ($p \le 0.05$) G1 and G2 technicians compared with controls. Whereas, G1 results decreased significantly at level ($p \le 0.05$) compared with controls and G2 results, while G2 values decreased significantly at level ($p \le 0.05$) compared with controls, but increased significantly at level ($p \le 0.05$) compared with G1.

No.	Group	Mean ±SE
1	Control	57.93 ±1.07
2	G1	$51.62 \pm 1.89^{*a}$
3	G2	$54.62 \pm 1.33^*$

Table (4-5): Neutrophil percentages obtained for all Cases and Controls

* refers to significant differences at level $P \le 0.05$. a refer to significant difference comparing with the other test group. $(M \pm SE)$: Mean \pm Standard error.

4.2.2.2 Basophil Counts

In general, basophils percentage for all cases involved in this research were ranged between (0-2%) with a mean of (0.4%). Control count for basophils were all

within normal accepted values. Normal ranges depended was (0-2%) (Lewis et al., 2011).

Peripheral blood Basophils percentages are viewed in table (4-6). There was a significant difference at the level of ($p \le 0.05$) G1 and G2 technicians results compared with control. Whereas, G1 and G2 results increased significantly compared with control. Also G2 mean percentage increased significantly compared with G1.

No.	Group	Mean ±SE
1	Control	0
2	G1	$0.23 \pm 0.13^{*}$
3	G2	$0.38 \pm 0.11^{*a}$

Table	(4-6):	Basoph	ils Perce	entages (Obtained	for all	Cases and	Controls
abic	(=-0)•	Dasoph		magus	optameu	iui an	Cases and	

*Refer to significant differences at level $p \le 0.05$

a refer to significant difference comparing with the other test group $(M \pm SE)$: Mean \pm Standard error.

4.2.2.3 Eosinophil Counts

Eosinophils results are illustrated in table (4-7), the results illustrated that there was no significant differences at the level of ($p \le 0.05$) between G1 and control results, while G2 values increased significantly at the level of ($p \le 0.05$) compared with controls', and G1 mean percentages.

Normal range depended was (0-5%) (Lewis *et al.*,2011). There were no elevated or decreased rates obtained for eosinophil in all cases involved in this study.

No.	Group	Mean ±SE
1	Control	0.17 ± 0.08
2	G1	0.09 <u>+</u> 0.06
3	G2	$0.52 \pm 0.12^{*}$

Table (4-7): Eosinophils Percentages Obtained for all Cases and Controls

*Refer to significant differences at level $p \le 0.05$ ($M \pm SE$): Mean \pm Standard error.

4.2.2.4 Monocytes Count

The result of monocytes percentages are shown in table (4-8). There were no significant differences among all groups. All values obtained were within normal accepted levels. Normal range of peripheral monocytes depended was (2-10%) (Lewis *et al.*,2011).

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No.	Group	Mean ±SE
1	Control	4.93 ±0.28
2	G1	5.66 ±1.03
3	G2	5.17 <u>±</u> 0.46

	Table ((4-8)): Monoc	vte Percentag	ge Obtained	l for all	Cases and	Controls
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 $(M \pm SE)$: Mean \pm Standard error.

4.2.2.5 Lymphocyte Counts

Lymphocyte results are shown in table (4-9); for both G1 and G2 the result showed significant increases at the level of ($p \le 0.05$) values in both groups compared with controls values, while there were no significant differences between G1 with G2 values. In general, there were (37) cases (61.7 %) from total (60) cases recorded elevated lymphocyte percentages above the high limit of normal range depended. Normal range (25-35%) (Lewis *et al.*, 2011).

Table (4-9): Lymphocyte Percentage Obtained for all Cases and Controls

No.	Group	Mean± SE
1	Control	35.37 <u>+</u> 0.60
2	G1	$41.90 \pm 1.87^*$
3	G2	$39.69 \pm 1.80^*$

*Refer to significant differences at level $p \le 0.05$ ($M \pm SE$): Mean \pm Standard error.

4.2.3 Hemoglobin Estimation

Technicians results ranged between (11.6-18.2%) for males and (9.9-16.2%) for females. All results for controls were obtained within normal range. There were (29) males (48.3%) showed increased values above the accepted normal reference with a consideration of smoking effects since all of them were smokers (Al-Hamadany *et al.*, 2016).

For statistical reasons, Hb% levels were analyzed without taking in account years of employment. The significance was taken for both gender mean value compared with controls.

There was no significant difference at the level of $(p \le 0.05)$ in the levels of Hb% for technicians males compared with the male levels of controls, similarly females results of technicians did not show significant differences at the level of $(p \le 0.05)$ when compared with control females values. Hemoglobin results are illustrated in table (4-10).

No.	Group		Mean±SE
1	Male	Cases	15.92 ± 0.17
		Control	15.95 <u>+</u> 0.35
2	Female	Cases	12.29 ± 0.57
		Control	13.89 <u>+</u> 0.46

Table (4-10): Hemoglobin Concentrations obtained for Cases and Contro	ols
Distributed by Gender	

 $(M \pm SE)$: Mean \pm Standard error.

4.3 Immunological Results

4.3.1 Results of NBT Test (Neutrophils Activity Results)

The NBT test is one of the best common selection tests for neutrophils function by phagocytosis. The reduction of yellow NBT dye; and creating insoluble Formazan blue crystal; indicating for the positive reaction, **picture (4-1)**.

NBT positive result showed a significant decrease in G1 values at the level of (p ≤ 0.05) compared with controls. Also G2 values decreased significantly at the same level compared with controls. Moreover; G2 values decreased significantly at the same level compared with G1 values.

Whereas, there were (51) cases (85%) from the total (60) X-ray technicians had levels below normal range. All controls recorded normal values, and no elevated value recorded in all technicians. Normal depended reference range was (60-75%) as in (Freeman and King, 1972&Al-Hamadany, 2011). The results of NBT reduction test are shown in table (4-11).

No. Group		Mean ±SE		
1	Control	70.20 ± 1.24		
2	G1	$55.00 \pm 2.72^*$		
3	G2	39.69 ± 1.80^{a}		

Table (4-11): Test NBT Percentages obtained for all Cases and Controls

* refers to significant differences at level $P \le 0.05$

a Refer to significant differences comparing with the other test group

 $(M \pm SE)$: Mean \pm Standard error



Picture (4-1): Heparinized Blood Smear for NBT Test, under Oil immersion X 100, ((a), (c) shows single cell reaction(positive result) and (d) showed negative result while (b) represent chemotaxis process).

4.3.2 Human Interleukin-2 (IL-2)

Interleukin-2 (IL-2) estimation results showed a significant decrease at the level of ($p \le 0.05$) in G1 values compared with controls and significant increase of G2 levels compared with controls at the same level. While G2 concentration of IL-2 increased significantly compared with G1 values.

Normal depended range was (15.6-1000) pg/mL as recommended by the manufacturing company of the kit used. Table (4-12) demonstrates the results of IL-2 levels means.

No.	Group	Mean ±SE
1	Control	26.41±4.59
2	G1	$24.82 \pm 4.10^*$
3	G2	$36.95 \pm 5.15^{*a}$

* Refer to significant differences at level $p \le 0.05$

a refer to significant difference comparing with the other test group $(M \pm SE)$: Mean \pm Standard error.

4.3.3 Human Interleukin 12 (IL-12) Estimation Value

There was no significant difference obtained for both G1 and G2 as shown in table (4-13) compared with control levels. Normal standard depended level range was (3.9-250) pg/mL. as in the leaflet provided with the kit and recommended by the manufacturing company. No elevated levels obtained nor decreased level in both cases and controls recorded.

No.	Group	Mean ±SE	
1	Control	195.11 <u>+</u> 0.19	
2	G1	195.39 <u>+</u> 0.4	
3	G2	195.26 <u>+</u> 0.34	

 $(M \pm SE)$: Mean \pm Standard error.

4.3.4 Human Interleukin 18 (IL-18) Estimation Value

Interleukin-18 result revealed significant increase at the level of ($p \le 0.05$) for mean values of both G1 and G2, comparing with control mean values; no significant differences were obtained G1 and G2 levels, **picture (4-2)**.

Normal depended range was (15.63-1000) pg/mL as recommended by the manufacturing company of the kit used for estimation. The results of IL-18 are demonstrated in table (**4-14**).

mL.)		
No.	Group	Mean ±SE
1	Control	56.81 <u>+</u> 5.38
2	G1	491.71 <u>+</u> 137.79 [*]
3	G2	692.07 <u>+</u> 93.75 [*]

Table (4-14): Interleukin-18 Levels Recorded for all Cases and Controls (pg/ mL.)

> *Refer to significant differences at level $p \le 0.05$ ($M \pm SE$): Mean \pm Standard error.

CHAPTER FIVE DISCUSSION

CHAPTER 5: DISCUSSION

The present study is the first study locally that focused light on X-ray technicians and their immune system health. According to the collected data, most of X-ray technicians involved in the study had no license to work in radiation field or in another word they are not undertaken a periodic checkup; they represented (60%) of the total technicians involved (**Table 4-1**). And the worst observation recorded was that almost a quarter of them (28.5%) were working in non-governmental clinic for X-ray photography for the end of their shifts in the governmental hospitals that caused a serious consequences on their immune system as the coming results illustrated.

In addition, there were no radiation protection equipment's supplied for X-ray technicians like (special gloves and gray lab coats) and even sometimes had no film badge, whereas there were only (48.4%) had film badge and nearly half of them, their film badges were old and not updated for long times.

Concerning the radiation protection center which is responsible for personal exposure monitoring of radiation observation in Iraq, the center refused to cooperate and support this study with its records and data about the overdosed cases in Al-Muthanna governorate. In the study of (Al-Hamadany,2011), the recorded exposed occupationals were 0.6% from the total licensed radiation occupationals in Iraq during the year 2006. While this ratio increased to (1%) during the year 2008.

5.1 Total W.B.Cs

There were no significant differences between G1 and G2 results with control's, and there were 15(25%) from the total (60) who recorded diminished levels compared with normal accepted values. This result was expected due to radiation exposure effects.

Meo, (2004) in his article classified X-ray technicians in groups, less than 5 year occupation and more than 5 years, he also found that there were no significant decrease obtained when comparing total W.B.Cs results with controls, but in general his research results showed a decrease in total W.B.Cs count due to radiation exposure, which is consistent with our result from the present study.

Mohammed *et al.*, (2014), and his colleagues studied the effects of X-ray exposure in technicians worked in different hospitals in Diyala/ Iraq. They found that there were no significant difference between occupational and controls and attributed that to the ability of hematopoietic system for fast recovery in short period upon radiation exposure and this opinion supports our results.

These finding are the same of Davondi *et al.*, (2012), who investigated hematological parameters change in radiation field workers blood and found that there were no significant differences between mean values of workers and controls despite of the decrease in W.B.C count individually, these finding are the similar to the present study.

5.2 Neutrophil Counts

Neutrophil result showed that acute exposure to X-ray as in G1and G2 group caused significant decrease in values but, the levels of G2 were increased significantly compared with G1. This result can be attributed to the acute radiation effects on the hematopoietic system (Lewis *et al.*, 2011), which included neutropenia as a symptom.

Radiation can cause a decline in granulocytes count after initial exposure as stated by (Prabhu *et al.*,2015).

In the study of (Al-Hamadany,2014), neutrophil relative numbers were decreased after exposure to radiation source during an *in vitro* irradiation of human blood experimentally.

Akleyev, (2014), presented chronic radiation syndrome symptoms and sorted out that neutrophils had the highest sensitivity cells among blood cells to radiation exposure, also he recorded a significant decrease in these cells relative numbers during both acute and chronic exposure to radiation but he stated that; with time when the radiation exposure become chronic the levels of neutrophils are recovered slightly but the phagocytic function still deficient. That is completely in agreement with our results.

Granulocyte colony stimulating factor (G-CSF) was suggested as a treatment for children with cancer receiving radiation therapy which causes Myelosuppression leading to neutropenia, a suggestion presented by (Marks *et al.*,1992). This scientist and his colleagues explained the decline in blood cells including neutrophils as a result of bone marrow suppression leading to decrease neutrophils production, especially neutrophils which are the most sensitive W.B.C to radiation exposure regardless of the dose of radiation, and that is consistent with the present study results.

5.3 Basophil Counts

Basophil results showed a significant increase in these cells relative number in technicians blood samples for both groups compared with controls'.

Moreover, G2 mean value increased significantly compared with G1. It is a scientific fact that basophils relative numbers increase during malignancy development and alteration, also exposure to ionizing radiation can cause elevation in basophil production. This opinion is supported by (Lewis *et al.*, 2011). Basophilia occurs during development of bone marrow disorders. Also,(Lamerton *et a.*,2014) stated that, long period of radiation exposure will lead to maintening some blood W.B.Cs component near normal levels. While (Joo *et al.*,2012) and his team investigate about ionizing radiation effects on mast cells (highly specialized basophils) found that, low dose of ionizing radiation can suppress mast cell activation and toxicity but has no effects on their relative number.

Al-Hamadany, (2011) investigated basophils relative count and found that the technicians in hospitals of radiotherapy and nuclear medicine had a significant increase in their peripheral basophils ratios comparing with controls' and that is in agreement with the present study.

5.4 Eosinophil Counts

Eosinophil results showed a significant increase in G2 group mean values at the level of $P \le 0.05$ compared with controls, meaning chronic exposure to X-ray caused a significant increase. Numan, (2007) carried out an investigation about eosinophilia and the results showed that chronic radiation by radiotherapy caused eosinophilia in patients, which is in agreement with the results of this study.

Also Hyeok Lim *et al.*, (2015) and his colleagues studied irradiation eosinophilia following radiotherapy for lung cancer cases, they found that radiation induced eosinophilia in lung alveoli.

5.5 Monocytes Count

Monocytes values for X-ray technician for both groups did not show significant difference according to controls. This may be due to the fact that these cells are the more resistant cells against radiation as stated by (Meckenzei, 2004) and (Lewis *et al.*,2011). This result was the same that obtained by (Al-Hamadany, 2011), whereas no significant difference was obtained on the ratios of monocytes for X-ray technicians working as radiotherapist. Meo, (2004) also found out that monocytes are resistant to radiation and unsusceptible for radiation in X-ray technicians.

5.6 Lymphocyte Counts

The results of lymphocytes showed that there was a high link between radiation exposure in X-ray technicians and lymphocytes ratios. There were significant increases in both mean values for G1 and G2 groups compared with controls.

Moreover there were 37 cases (representing 61.7 %) from total cases in this study recorded elevated values that exceeded normal high limit. That can be attributed to radiation interference and effects on these important cells. These results are supported by (Mohammed *et al.*, 2014) in a study carried out; in Baqubah teaching hospitals/ Iraq; on X-ray technicians, they found that lymphocytes were the most affected cells in the hematopoietic system, they recorded significant aberration and abnormal morphology among lymphocytosis cases of X-ray technicians.

Rozaj *et al.*, (1999) stated that lymphocytes recover fast after radiation exposure and rise in number and may be produced in high intensity compared with other hematopoietic cells after radiation exposure. This opinion supports our findings.

Paretzke, 1997 and his colleagues discussed radiation exposure effects on human body and listed lymphocytes on the most affected blood cells by radiation exposure and an induction of these cells from bone marrow following exposure is a state usually recorded after irradiation, that is also the same point that (Chukhlovin *et al.*, 1994) discussed and suggested that radiation induced post exposure recovery process for blood system by stromal functional activation.

5.7 Hemoglobin Estimation

Hemoglobin percentages results illustrated that there was no significant difference between controls and cases obtained values regarding genders. These results can be

CHAPTER 5: DISCUSSION

explained by the fact that erythrocytes are radio resistance cells in blood as stated by (Lewis *et al.*, 2011 and Meckenzei, 2004).

The authors (Thomas, 2008) investigated X-ray effects by irradiating human blood. They found that RBCs did not show differences after irradiation suggesting that these cells are resistant against gamma radiation. Also, fast recovery of hematopoietic system post exposure has significant role in restoring normal Hb% values for technicians. This opinion is also supported by (Hoffbrand *et al.*, 2006).

In the previous study of (Janatpour, 2005), it was found that RBCs membrane permeability after irradiation with X-ray will suffer from differences which are not to be clinically important, and that is consonant with the results of this study.

5.8 NBT Test (Neutrophils Activity Results)

Neutrophils function was obviously diminished as the present study results showed, all technicians mean values decreased significantly with an obvious relation to radiation exposure.

Moreover, chronic radiation effects were more intense compared with acute as the significancy of the decrease in mean values for G2 group results was more compared with G1group mean value, and that can be a result of radiation accumulation in human body upon continuous exposure to X-ray during work.

These results are supported by many previous, studies such as (Thomas and Cardigan, 2008), who found that neutrophils function were suppressed by irradiation when X-ray was applied on human blood sample.

Meo (2004) stated that neutrophils phagocytic activity for X-ray technician was significantly decreased compared with controls and attributed that to radiation effects on free radicals system of these cells used for killing pathway during phagocytosis process.

Akleyev(2014) explained chronic radiation effects on neutrophils and monocytes by effecting lysosomal component leading to suppressed phagocytic function, and that is in agreement with the findings of the present study.

Al-Hamadany, (2014), found that irradiation of neutrophils in human blood samples caused suppression in NBT reduction ability hence phagocytic function inhibition and attributed that to early apoptosis induction.

5.9 Human Interleukin-2 (IL-2)

Interleukin-2 results showed that acute radiation exposure for X-ray occupationals represented by G1 group members caused a significant decrease in IL-2 levels compared with controls, while IL-2 levels increased significantly in chronic X-ray exposure as in G2 group levels compared with normal (represented by controls) and acute exposure represented by G1 levels.

Manda *et al.*, (2012) and his team stated that irradiation of whole human body caused acute irradiation syndrome and usually leads to suppression of immune function due to alteration of cytokines release; like IL-2 increase in levels.

Dainiak, (2002) in his investigation about radiation exposure effects on hematologial components reported that IL-2 production by T-cells is reduced in

CHAPTER 5: DISCUSSION

atomic bomb survivors after ionizing radiation exposure, these facts are supporting our results since acute exposure in G1 caused significant elevation in IL-2 levels.

Interleukin-2 is the most important immune response modulating factor as stated by Xu *et al.*, (1996), when they studied IL-2 production by lymphocytes of radiation occupational blood samples and they found that this interleukin levels increased immediately after radiation exposure.

Al-Hamadany *et al.*, (2011), demonstrated that acute exposure to radiation influenced X-ray technicians and caused a significant increase in IL-2 levels. These results are consonant with the results of the present study.

On the other hand; (Sonn *et al.*,2012) and his colleagues concluded that chronic irradiation effects of ionizing radiation exposure caused increase in natural killer cells cytotoxicity due to IL-2 levels increase in response to chronic radiation exposure. These findings support the opinion of chronic IR exposure caused a significant elevation in IL-2 levels of G2 group.

Another important study accomplished by Zakeri, (2010) on X-ray radiation biological effects on medical staff, they found that IL-2 levels significantly increased upon continuous exposure due to increase in aberrant cells frequencies in these occupational blood. These are similar to our findings.

5.10 Human Interleukin 12 (IL-12)

Result of IL-12 did not show a relationship between X-ray exposure and this cytokine levels since no elevated nor decreased levels were recorded in both groups G1 and G2. All results were within normal accepted values and moreover no significant difference was obtained in test groups and controls.

Despite of the fact that IL-12 is the cytokine that is responsible for differentiation of T-cell into Th1 and usually increased during Th1 immune response. The present study result showed different results, Dong Lui *et al.*, (2003) found that IL-12 expression increased upon irradiation of mice with X-ray, also Di Maggio *et al.*, (2015) investigated different cytokines production and expression in response to ionizing radiation; the increase was due to radiation induction of this cytokine expression.

Another opposite opinion was stated by (Merrick *et al.*,2005), who concluded that irradiation caused reduction in IL-12 production furthermore, Geber *et al.*, (2015), and his team suggested IL-12 as treatment for avoiding radiation exposure damage on immunological barriers in skin. Ghazy, (2015) and other scientists concluded that IL-12 levels can differ post gamma radiation exposure in dose dependent manner, whereas, IL-12 levels increased in low dose of radiation and decreased in higher dose, and that is supporting our results.

The present study result of IL-12 normal levels may be linked to monocytes results, since there were no significant change recorded on these cells in the groups under testing whereas, these cells are the producer of this interleukin.

5.11 Human Interleukin 18 (IL-18)

Interleukin-18 results showed a strong relation to radiation exposure since this cytokine levels increased significantly in test groups blood samples compared with controls.

The scientist Ha *et al.*, (2014), suggested using IL-18 as a biomarker for radiation exposure since its levels increased significantly in laboratory animals after irradiation in a dose dependent manner and related to radiation injury severity. Other supportive results were obtained by Yang *et al.*, (2006), who investigated the relation between X-ray irradiation and IL-18 levels in mice and found that IL-18 levels increased in macrophages after irradiation with both high and low-doses of X-ray.

Shan *et al.*, (2007) stated that ionizing radiation induced IL-18 secretion by activation of its production pathway in macrophages of mice, which is consistent with present study results.

These result may be related to this cytokine role as antitumor factor during CMI, many scientists suggested this interleukin as a therapy for cancer patients because its activity during cancer fighting and altered cells resulted from IR exposure. (Tian *et al.*, 2014) prepared a vaccine as gene therapy when they transfected lung cancer cells with a cloned plasmid liposome containing IL-18 producing genes, they found after irradiation that this vaccine induced antitumor efficacy by enhancing cytotoxic activity of NK cells, moreover cancer cells stopped their proliferation process.

Singh *et al.*, (2016), investigated the biomarkers for radiation injury and they found that IL-18 levels increased after irradiation with γ -radiation showing a direct correlation with the absorbed dose in experimental animals.

Gamma radiation induces changes in the surface receptors of the influential cells in the immune system including macrophage, dendric cells and lymphocytes due to chromosomal aberration, mainly during chronic radiation exposure hence altered cells appear in high frequencies. Moreover, these changed cells will be either in early apoptosis status or unfunctional cells not able to receive or send messages; by secretion or induction; via cytokines that modulate immune responses in human body. Akleyev, (2014) found that the immune system suffered from suppression and weakness and many recurrent infections and cancerous cells appeared, mostly in Xray technicians of long occupation period in radiation field. This result was illustrated and discussed also by Akleyev, (2014) in his research on radiation exposure consequences on human body and the results he discussed were very similar to our findings and the opinion he presented were supporting to our result in that IL-18 is the most appropriate cytokine to be tested for people with IR exposure.

CHAPTER SIX CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

1. Innate immunity of X-ray technicians, represented by phagocytic activity and other related parameters; was highly affected by this type of IR exposure.

2. X-ray exposure caused suppression and lost regulation of CMI due to improper communication among immune cells involved in CMI and that was obviously seen in the disturbance of IL-2 and IL-18 levels.

6.2 Recommendations

1. Periodic checkup for X-ray technicians in Al-Muthanna governorate should be done, observed and updated especially for X-ray technicians who work in non-governorate X-ray clinics.

2. Addition of the critical cytokines (IL-2 and IL-18) and phagocytic activity detection in the periodic checkup tests, to give a view for CMI status for X-ray technicians.

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APPENDICES

Appendix A

Periodic Medical Examination Form for radiation field occupationals, taken from Gynecology and Pediatric Teaching Hospital. Al-Muthanna Governorate.

<i></i>	ك اله قابة من الاشعاع		
التأثير البيولوجي	ر الشخصي / شعبة در اسبة ا	قسم مراقبة التعرض	(
، حقل الإشعاع	، الطبي الدوري للعاملين في	أستمارة الفحص	
الجنس	تاريخ الولادة		لاسم الرباعي واللقب
، العلمي با سابقاً	المؤهل اسم المؤسسية التي عمل ب	الذي يقوم به ما دما حاليا	لمهنية وطبيعة العمل اسم المؤسسية الترري
رقم الموبايل	رقم الفلم	ىۋىسىية	ل زود بغلم وفي أي
ن معنین بینین ادار	لفخص البريد (بسرونم محنة	،	تاريخ ترويده باول قد عنوان المؤسسة
مسات المدرجة أدناه تكون فحوصاتهم كل	ملين في حقول الإشعاع ما عدا المؤ	، الفحوصات الدورية سنويا للعا	بلاحظة • يكون إحرا
•			<u>ستة اشهر</u>
	سل	ن الإستعاع والطب اللووي /بعداد ن الأورام والطب التووي / الموه	۱. مستشفر ۲. مستشف
	ع النظائر المشعه المفتوحه .	مؤسسات الأخرى التي تتعامل م المقاربة معالاة مام	٣. جميع ال
		الوقاية من الإشفاع	يظات طبيب مركز
Blood examination.			مم المحلل توقيع
Hb	gm / dl.		ريجي
P.C.V	%		
W.B.C	$\times 10^{9}/L.$		
Platelets count	$\times 10^9$ /L		
ESR	mm/ hr.		
Differential count			
Neutrophils			
Lymphocytes	%.		
Monocytes	%.		
Eosinophils	%.		
Basanbile	%.		
Abnormal and	%.		
Pland Film	%.		5
<u>Blood Film</u> :		н 	
معادة ومروقة تعاد الاستعادة		ص :-	توفيع وختم المحا

Appendix B



	bioelisa reader	EL×800
ssay:IL- 2	Date:14/03/16	Lot: Operator: Plate ID:
avelength:405	Temp:	

)MMENTS

.

< > around RSLT indicate extrapolated concentration.



الاقرار

نحن نشهد كأعضاء لجنة مناقشة قد اطلعنا على هذه الرسالة الموسومة ب (تأثير الأشعة السينية على بعض المعايير المناعية في العاملين في الاشعة التشخيصية في محافظة المثنى) وقد ناقشنا الطالبة (مروة حسين محل) في تاريخ ٢٠١٧/٩/١٢، ووجدنا بأن الرسالة تفي بمستوى الحصول على درجة ماجستير علوم/ علوم حياة / أحياء مجهرية.

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أ.م. د حسين جابر عبد الحسين

عميد الكلية

الخلاصة

سلطت هذه الدراسة الضوء على صحة الجهاز المناعي لدى تقنيى الأشعة السينية، حيث ان الأشعة السينية هي نوع من انواع الاشعاع المؤين ذو الطاقة العالية والذي ينتج شحنات كهربائية عند مروره في المواد. ان التعرض لمثل هذا الاشعاع يسبب العديد من السرطانات و الأخماج المتكررة، ولأن العاملين بالأشعة السينية في تعرض مستمر للإشعاع فان تقييم صحة جهازهم المناعي تم من خلال قياس بعض المعلمات، وتعد هذه الدراسة هي الاولى من نوعها في محافظة المثنى. تم جمع (٨٩) عينة تضمنت (٦٠) حالة (تقنيين عاملين بالأشعة السينية) و(٢٩) أشخاص اصحاء، اخذت عينات الدم خلال الفترة من كانون الاول ٢٠١٥ حتى اذار ٢٠١٦ من مستشفيات محافظة المثنى (الحسين التعليمي و الولادة والاطفال و الرميثة و الخضر) ومن المركز التخصصي لطب الاسنان هذا فيما يخص العاملين بالأشعة السينية، اما عينات السيطرة فقد اخذت من اشخاص اصحاء ضمن مجتمع محافظة المثنى ممن ليس لهم اي صلة او تعرض سابق للإشعاع. اهتمت الدراسة الحالية بالتحري عن معلمات ذات ارتباط مباشر بالتعرض للإشعاع حيث تضمنت العد الكلي لكريات الدم البيضاء Total W.B.Cs count و العد التفريقي لكريات الدم Differential W.B.Cs و قياس تركيز هيمو غلوبين الدم و اختزال صبغة NBT لقياس مستوى الفعالية البلعمية للكريات العدلة و فحص ELISA لقياس مستوى الوسائط الخلوية L-12 و IL-18 و IL-18، بالإضافة لذلك فانه قد تم تسجيل المعلومات الضرورية لكل حالة. اظهرت النتائج بوجود تثبيط في المناعة الطبيعية و المناعة الخلوية Innate and cellular Immunity لدى العاملين بالأشعة السينية بسبب اضطراب المعلمات ذات الصلة بالتعرض للإشعاع سواء الحاد او المزمن. بالإضافة الى ذلك فان البيانات التي تم تسجيلها اظهرت فقر التجهيزات و المعدات ذات الحماية الشخصية PPE اضافة الى ضعف الفحص الدوري و الرقابة على التعرض الشخصي للإشعاع للعاملين في الاشعة السينية في محافظة المثنى.



جمهورية العراق وزارة التعليم العالي والبحث العلمي جامعة المثنى كلية العلوم

تأثير الأشعة السينية على بعض المعايير المناعية في العاملين في الاشعة التشخيصية في محافظة المثنى

رسالة مقدمة إلى مجلس كلية العلوم/ جامعة المثنى كجزء من متطلبات نيل درجة الماجستير علوم/ علوم حياة/ أحياء مجهرية

> من قبل مروة حسين محل بكالوريوس علوم حياة ٢٠١٣

> > بإشراف أ.م.د. وئام سعد الحمداني